On the Estimation of Total Behavioral Repertories in Ants

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Abstract: The total behavioral catalog size of *Leptothorax curvispinosus* workers in a nest environment has been estimated by means of the Fagen-Goldman method of fitting frequency data to negative binomial and lognormal Poisson distributions. The worker repertory is characterized by a smaller number of rare behaviors in comparison with vertebrate repertories. This trait makes the preparation of an adequate ethogram much less time-consuming. The behavior of a partially bilateral worker-male gynandromorph is described and the estimation method used to show that its repertory is probably intermediate in size between those of full workers and males. The limitation of worker behaviors to the worker (as opposed to male) appendages suggests a bilateral as opposed to diffuse control of movement by the gynandromorph's brain. The advantages and difficulties of the estimation technique are then discussed.

INTRODUCTION

The listing of behavioral repertories to produce "ethograms" is an essential first step in the comparative study of behavior. But it is also one of the most time-consuming. Studies of single bird species commonly last hundreds of hours, while a few primate projects have consumed a thousand observation hours or more over a period of years. Even at this level, there has been no systematic way of judging how nearly complete the ethogram has become, and ethologists have ordinarily relied on unaided intuition in choosing the time to stop. Recently Fagen and Goldman (1974) proposed a method for estimating the total size of behavioral categories by fitting frequency data of behavioral acts to one or both of the most general distributions likely to be appropriate, namely the lognormal Poisson and negative binomial.

The present article examines the application of this technique to two castes of the ant genus *Leptothorax* and considers its general strengths and weaknesses for insect studies. The method has also been used to evaluate the repertory of a rare gynandromorph discovered in a colony of *L. curvispinosus*.

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Table 1. Relative frequencies of behavioral acts by workers and a gynandromorph of the ant *Leptothorax curvispinosus* and by males of *L. duloticus*. (N, total number of behavioral acts recorded in each column)

Behavioral Act	*Leptothorax curvispinosus* workers ($N = 1962$)	*L. curvispinosus* gynandromorph ($N = 45$)	*L. duloticus* males ($N = 65$)
1. Self-grooming	0.2370	0.7333	0.6462
2. Antennal tipping	0.0122	0	0
3. Allogroom worker	0.0428	0.0667	0
4. Allogroom queen	0.0025	0	0
Brood care:			
5. Carry egg	0.0153	0	0
6. Lick egg	0.0255	0	0
7. Carry larva	0.1264	0	0
8. Licking larva	0.1804	0	0
9. Assist larval ecdysis	0.0056	0	0
10. Feed larva solid food	0.0336	0	0
11. Carry pupa	0.0122	0	0
12. Lick pupa	0.0484	0	0
13. Assist eclosion of adult	0.0082	0	0
14. Lay egg	0.0025	0	0
Regurgitate:			
15. With larva	0.0775	0.0222	0
16. With worker	0.0642	0.1778	0.3538
17. With queen	0.0138	0	0
18. Fight queen or workers	0.0092	0	0
19. Lick wall of nest	0.0138	0	0
20. Forage	0.0291	0	0
21. Feed on honey	0.0056	0	0
22. Feed on solid	0.0173	0	0
23. Carry dead insect	0.0025	0	0
24. Carry dead nestmate	0.0025	0	0
25. Carry live nestmate	0.0015	0	0
26. Handle nest material	0.0041	0	0
27. Stridulate	0.0061	0	0
TOTALS	1.0	1.0	1.0

METHODS

Colonies of *Leptothorax* were collected by Dr. Mary Talbot at the E. S. George Reserve, near Pinckney, Michigan. Some consisted of pure *L. curvispinosus*, with *curvispinosus* queens, others of *curvispinosus* enslaved by the rare parasitic species *L. duloticus*, the latter containing *duloticus* queens. The colonies were maintained in narrow glass tubes moistened by cotton wool at one end and left open at the other. The workers were allowed to forage freely out of the tubes and onto the floor of small, steep-sided containers. The containers were small enough in turn (9×15 cm on the side by 6 cm deep) to be placed on the stage of a dissecting microscope. As a consequence the entire worker populations of colonies, consisting of 20 to 100 workers, could be monitored simultaneously. Behavioral catalogs were constructed and frequencies of each behavior accumulated by scanning back and forth for as long as an hour or more in continuous sessions. By this means it was possible to record all of the discrete behavioral acts displayed by virtually every worker. Observation periods were scattered according to convenience from 8 in the morning to one or two hours past midnight. Over this span no differences in level or pattern of activities were noted. Nor were any expected, since the internal nest environment remained essentially constant.

Behavioral repertories and their frequency distributions did not differ significantly between pure *curvispinosus* colonies and those mixed with *duloticus*. Consequently, in order to obtain as large a sample size as possible, counts were taken from two *curvispinosus* colonies that had been especially well analyzed in connection with a separate study of ant slavery (see Wilson, 1974), one pure and the other enslaved. When a worker-male gynandromorph eclosed in one of the pure colonies during the course of the study, it was closely monitored during its short life. Simultaneously, *duloticus* males in a mixed colony were monitored; *curvispinosus* males were not available at this time for quantitative study, but earlier studies had shown that the repertories, if not the frequency distributions, were identical. The data were then analyzed by the method of Fagen and Goldman.

RESULTS

Worker repertory. The behavioral catalog and frequency data are presented in Tables 1 and 2 and Fig. 1. The estimates given were based on a fit of the data to the negative binomial distribution. Similar results were obtained with the lognormal Poisson distribution. In the case of *L. curvispinosus* workers, the estimated total repertory size is 29, with a 95 percent confidence interval of [27,35]. The sample coverage, defined as $\sum_i p_i$, where p_i is the probability of performance of each observed act i, is much greater, being 99.95%. This very high value means that the still missing behaviors have an aggregate probability of 0.0005.

Male repertory. The repertory of the *L. duloticus* males in the nest was extremely limited, and the two behaviors observed were not far from equiprobability. As a result the estimated repertory is identical to the observed repertory, a remarkable result in view of the small number of data utilized.

Male-worker gynandromorph. The observed repertory falls far short of the estimated repertory, especially the upper limit of the 95 percent confidence interval, a result that accords well with our intuitive feeling during the period

Table 2. Catalog and estimated total repertory of two castes and a worker-male gynandromorph of *Leptothorax*. Estimates were obtained by fitting the data of Table 1 to a negative binomial distribution

	No. of acts observed	No. of kinds of behaviors observed (observed repertory size)	Estimated total repertory size	Estimated 95% confidence intervals, total repertory size
L. curvispinosus workers	1962	27	29	[27,35]
L. duloticus males	65	2	2	[2,2]
L. curvispinosus gynandromorph	45	4	7	[4,27]

of observation. Because opportunities seldom arise for the observation of living gynandromorphs, further notes on this one individual will now be given. Data were taken on an almost daily basis from the time the ant was discovered as a one- or two-day-old callow until its death eleven days later. The total observation time was six hours.

The gynandromorph was the size of a small worker. The body behind the head was covered preponderantly by worker exoskeleton (easily distinguished by its yellow as opposed to blackish brown coloration). The only male portions were the left lateral edges of the pro- and mesothoraces and the left fore and middle legs. The two legs were mostly useless, ordinarily being carried folded beneath the body. We gained the impression that these two appendages, which were longer and more slender than their worker counterparts, were under the control of the worker part of the central nervous system. (Presumably the thoracic ganglia consisted of worker tissue.) The foreleg often moved in a nearly normal fashion down to about the level of the metatarsus, where a worker leg would have ended, but the terminal segments kept folding under the metatarsus when the leg was moved forward and down.

The division of the head was exactly bilaterally symmetrical. So precise was the line of demarcation that the median ocellus was half developed—on the male side. It is a reasonable supposition that the division extended through the brain. The behavior of the gynandromorph proved to contain an interesting mixture of male and worker elements, as follows.

1. *Level of activity.* The gynandromorph was quite inactive and spent most of its time resting in one position. In this respect it much more closely resembled males of the same age than workers. When nearby workers discovered honey and began to rush excitedly in and out of the tube nest while regurgitating to

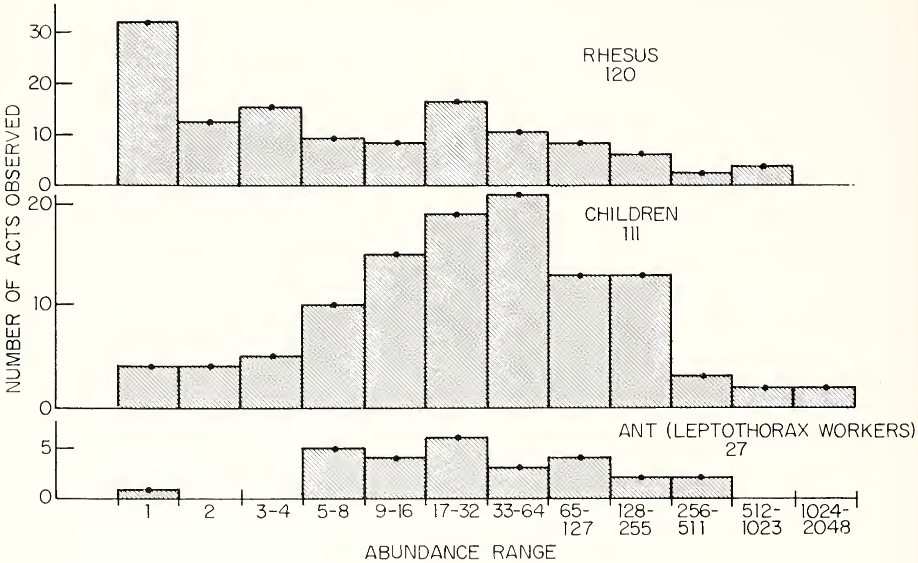

FIGURE 1. Frequency distributions of the 27 observed behaviors of *Leptothorax curvispinosus* workers, 111 behaviors of playing children, and 120 behaviors of rhesus monkeys. The mode has clearly emerged in the ants and children, indicating that most kinds of behaviors have been cataloged. This is particularly true of the ants, in which relatively few rare categories have so far been discovered. (Human and rhesus data from Fagen and Goldman, 1974.)

each other, the gynandromorph did not participate, a male-like rather than worker-like characteristic.

2. *Location.* The gynandromorph spent over 90 percent of its time at all hours of the day standing or walking around slowly within one cm of the nest entrance. This was a position sometimes taken by young workers but seldom if ever by males, which preferred to remain deep in the nest and especially near the brood. Males showed a circadian increase in activity, sometimes walking all the way out of the nest and attempting to escape from the foraging arena between about 9 PM and 1 AM. Males from wild colonies were captured at lights at 11 PM. Together, these data indicate that nuptial flights are conducted at night. No such circadian rhythm was noted in the gynandromorph.

3. *Allogrooming.* On three occasions the gynandromorph was observed to groom workers, a behavior commonly seen in workers but not in males. Significantly, the worker antenna was employed for orientation much more than was the male antenna during these bouts. On another occasion the gynandromorph regurgitated with a larva, another behavior characteristic of workers but not of males. The worker antenna was used to investigate and the worker fore

tarsus to stroke the larva; the corresponding male appendages were not employed. Although the allogrooming responses were worker-like, they occurred less frequently than in full workers.

4. *Antennal posture and general orientation.* The male antenna was held in a more extended position than the worker antenna; the postures of both were typical of the caste they represented. When the gynandromorph investigated a worker nestmate (as opposed to grooming it), both antennae were used equally.

5. *Investigation of solid food.* The gynandromorph was seen to explore a fragment of moth thoracic muscle being eaten by a larva, a behavior common in workers but not seen in males. During this brief episode only the worker antenna was used.

In summary, the gynandromorph displayed a mixture of male and worker traits. Its actual and estimated total repertory sizes were intermediate between those of males and workers. The estimation technique indicates that a smaller fraction of the total repertory was observed than in the case of the full males and workers. The worker behaviors were also displayed less frequently than in full workers. When the gynandromorph behaved as a worker, it used its worker antenna primarily or exclusively, suggesting a bilateral separation of effector control at the level of the central nervous system as opposed to a mixed control. This correspondence between anatomical and behavioral mosaicism is consistent with earlier findings on *Drosophila* and *Habrobracon* (Manning, 1967; Stern, 1968; Hotta and Benzer, 1972).

DISCUSSION

Let us next consider the strengths and weaknesses of the Fagen-Goldman catalog estimation method with special reference to ants and other insects. The obvious advantage of the technique is that it improves unaided intuition without forcing any new, unsupportable assumptions on the analysis. It is possible to judge more precisely the point of diminishing return during the preparation of ethograms.

This point came surprisingly early in the case of the ants. After only 51 hours of observation, during which 1,962 separate acts were recorded, the mode of the frequency curve emerged and the estimated sample coverage attained 99.95 percent. Thus the effort required to secure a nearly complete repertory seemed to be a full order of magnitude less than in the vertebrates. This result implies that comparative ethological studies can proceed much more rapidly in ants and other insects.

A reason of considerable potential biological interest exists for this relative tractability of ant studies. This is the scarcity of rare acts compared with common acts (see Fig. 1). In other words, whatever ants do they do rather frequently; few if any rare behaviors exist to surprise the investigator in the late stages of a study. We conjecture that the small size of the ant brain precludes the storage of responses that are not used commonly. As one of us has pointed out previously (Wilson, 1971), a characteristic of behavior in social insects is the repeated use of the same communicative signals and responses in different contexts to achieve various purposes.

There are two disadvantages of the method which we do not regard as particularly serious. The first is the probability that the repertories and frequency distributions change in different contexts. It remains for the biologist to define those contexts and to repeat the analysis within them. In the case of ants distinguishable contexts are not only finite but also probably quite limited in number. By far the greatest part of an ant's life is conducted in the homeostatic environment of the nest interior. Thus the lifetime sample coverage in the present study, defined as the cumulative probability of all behavior for all contexts, was probably very high in spite of the fact that it was limited to one environment. We suggest that the following list might exhaust the remaining contexts for the worker caste: extended foraging periods; major disturbances of the nest, including invasion by alien colonies, flooding, and overheating; emigration to a new nest site; and assisting during the initiation of nuptial flights on the part of the reproductive forms.

The second difficulty in repertory estimation is the arbitrariness of the definition of the behavioral act. One observer might see three distinct neuromuscular patterns where another sees only one. Thus "foraging" as defined in the present study could easily be broken down into several acts. This is essentially a problem of language, and different observers can solve it by a straightforward mapping procedure. One observer's acts *a, b,* and *c* will be recognized as comprising the second observer's act *a*; the first observer's act *h* will be seen as representing the second observer's acts *m* and *n*; and so forth. No great difficulty should occur when the same species is considered or closely related species are compared. Serious conceptual problems might exist, however, when an attempt is made to compare the size and frequency characteristics across radically different species.

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